A Phenotype Resembling the Clouston Syndrome with Deafness Is Associated with a Novel Missense *GJB2* Mutation

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Mutations in *GJB2* (connexin26) are associated with skin disorders and deafness. The Clouston syndrome (MIM129500) is associated with mutations in *GJB6* (connexin30). Here, we describe a patient suffering from a Clouston-syndrome-like phenotype of thin hair, deafness, nail dystrophy, and mild erythrokeratoderma, caused by a novel spontaneous missense mutation in *GJB2*. The heterozygous mutation in codon 42, AAC > AAG, changes asparagine to lysine (N14K). Interestingly, this asparagine is near two of the residues mutated in Keratitis-like ichthyosis deafness (KID) syndrome (G12R and S17F), yet the phenotype associated with N14K strongly differs from the KID phenotype. Instead, there is clear phenotypic overlap with syndromes associated with connexin26 or 30 mutations. Our findings suggest that careful audiological evaluation of patients suffering from Clouston-syndrome-like phenotypes is warranted and expand the spectrum of connexin26-associated disease.

Key words: Clouston syndrome/connexin26/gap junction/*GJB2*/hypotrichosis
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Gap junctions are intercellular communication channels that are vital for cell growth and differentiation in various tissues. They consist of connexins, relatively small proteins that belong to an extensive protein family that exist throughout the vertebrate kingdom. During the past years, several often syndromic skin disorders have been shown to be associated with mutations in the skin-expressed connexin genes *GJB2*, *GJB3*, *GJB4*, and *GJB6* (Richard, 2000; Kelsell *et al*, 2001). Sensorineural deafness is a frequent component of these disorders that otherwise have widely differing phenotypes. One of the most intriguing gap junction genes is *GJB2* (connexin26, Cx26) that has been implicated in several different disorders such as non-syndromic sensorineural deafness, palmoplantar keratoderma with deafness, and keratitis (and hystrix-like) ichthyosis deafness (KID/HID) (Kelsell *et al*, 2001; van Geel *et al*, 2002; van Steensel *et al*, 2002). We now report a novel connexin26 mutation that expands the spectrum of disorders associated with mutations in *GJB2*. The phenotype associated with this novel mutation resembles that of the Clouston syndrome (MIM129500), which is usually associated with mutations in *GJB6* (Lamartine *et al*, 2000; Smith *et al*, 2002). Interestingly, the mutation affects an asparagine that neighbors amino acids associated with KID syndrome.

The patient, a 2-y-old girl, was born to non-consanguineous Dutch parents. There are no other sibs and the family history was wholly unremarkable. Shortly after birth, it became clear that the child suffered from severe bilateral hearing loss that was later classified as sensorineural. Atopic eczema was said to have appeared directly after birth. Around age 6 mo, she fell ill and started refusing food. In addition, a slight developmental delay was noted at the time. At age 1, two episodes of viral enteritis led to dehydration, necessitating hospitalization twice. At two occasions during hospitalization, pronounced redness and swelling of the oral mucosa and gingiva were noted. The patient was referred to our department for diagnosis of her skin and nail problems.

Upon examination, we noted mild scalp hypotrichosis with lank, blonde hair. On the scalp some sharply demarcated erythematous plaques with some desquamation were present. In the neck we saw a papular exanthema but no other signs of atopic eczema. The patient displayed peculiar skin reactions upon application of brown adhesive bandaging (Fig 1*a*), consisting of sharply demarcated red plaques. The lesions resolved spontaneously with some scaling. Slight frontal bossing with seemingly deep-set eyes and 20-nail dystrophy were seen (Fig 1*b–c*, feet not shown). No other abnormalities were present except for mild perianal erythema. We could not substantiate the finding of excessive redness and swelling of the oral mucosa. The parents said that their daughter occasionally suffered from severe itching. Sweating was normal. The diagnosis presented some difficulty since the occurrence of nail dystrophy with hypotrichosis suggests the Clouston syndrome. Deafness, however, is not normally part of the Clouston syndrome phenotype. We considered the possibility of a novel variant of the Clouston syndrome, possibly associated with a *GJB6* mutation and initiated connexin mutation screening.

Abbreviation: KID, keratitis-like ichthyosis deafness

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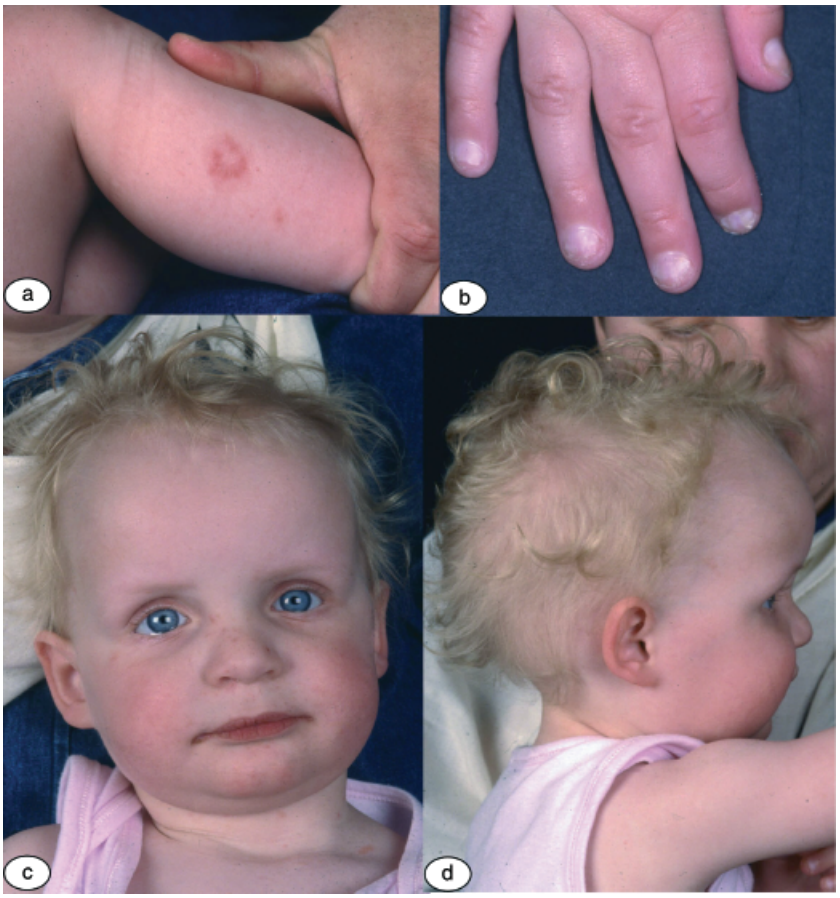

Figure 1
(*a*) Erythematous skin lesions arising after application of a brown adhesive bandage. (*b*) The fingernails are dystrophic. (*c* and *d*) The patient's phenotype. Note the frontal bossing in (*d*) and seemingly deep-set eyes. The hair is thinly implanted.

Informed consent was obtained from the parents. DNA was isolated from peripheral blood lymphocytes using protocols described elsewhere (Miller *et al*, 1988). We amplified the coding regions of the skin-expressed connexin genes *GJB2*, *GJB3*, *GJB4*, *GJB5*, and *GJB6* (connexin26, 31, 30.3, 31.1, and 30, respectively) by PCR. The PCR fragments were subjected to direct sequencing as previously described (van Steensel *et al*, 2002). The mutation analysis was repeated in an independent laboratory. Screening of 96 healthy controls for the nucleotide

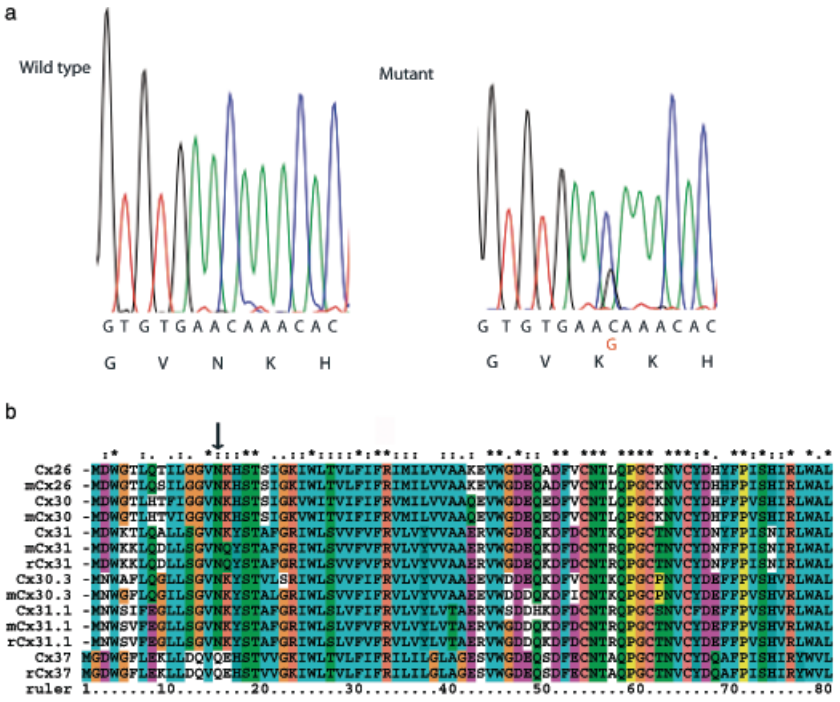

Figure 2
(*a*) Sequence traces showing the 44 C>G transversion mutation resulting in the substitution of asparagine 14 for a lysine. (*b*) CLUSTALX alignment of connexins from different mammalian species showing conservation of N14 (m = mouse, r = rat, h = human).

variation was carried out by *Mbo*II restriction analysis of the PCR products, where the mutated allele generates a site for digestion. In *GJB3*, *GJB4*, *GJB5*, and *GJB6*, no mutations or known polymorphisms were detected. In *GJB2* (connexin26), we found a heterozygous 44C > G transversion at codon 42), resulting in a substitution of an asparagine by a lysine (N14K, see Fig 2*a*). This asparagine is located at the intracellular C-terminus of the protein and is conserved among connexins (Fig 2*b*). The mutation was not found in either parent or in 192 unrelated control alleles from the Dutch population (with *Mbo*II restriction analysis). To our knowledge, this missense mutation has not been previously described in the literature.

Our results suggest that a phenotype resembling the Clouston syndrome but associated with deafness can be caused by a novel heterozygous mutation in *GJB2*. The Clouston syndrome has thus far only been associated with mutations in *GJB6*. Intriguingly, although most *GJB2* mutations described to date are associated with unique phenotypes, the N14K substitution that we found leads to a phenotype that is remarkably similar to the Clouston syndrome. This may be explained by involvement of residue N14 in heterotypic connexon assembly. If mutated it may interfere with the incorporation of *GJB6*; however, the Clouston syndrome is not associated with absence of *GJB6*, therefore this hypothesis seems unlikely. Alternatively, some missense mutations in both connexins are known to lead to disturbed gap junction conductivity (Rabionet *et al*, 2000). Perhaps the N14K substitution has an effect on heterotypic connexon assembly and results in faulty gap junction function that is comparable with the Clouston syndrome-associated *GJB6* mutations G11R and A88V. In addition, part of the phenotype may be explained by a trans-dominant effect of the *GJB2* mutation on *GJA1* and *GJB6* expression (Rouan *et al*, 2001). The associated phenotype in our patient suggests that the effect of the Clouston syndrome *GJB6* mutations is mediated at least in part by a similar mechanism. But neither trans-dominant effects nor disturbance of gap junction assembly and conductance can fully account for the phenotypes associated with *GJB2* mutations. For instance the mutations in two different domains of the protein in KID/HID syndrome (G12R/S17F and D50N) and the patient described here (N14K) show that mutations in residues located close together can result in radically different phenotypes. KID/HID syndrome is associated with severe bilateral early-onset keratitis, severe erythrokeratoderma, hypotrichosis, and a propensity to develop squamous cell carcinoma of the skin. Our patient on the other hand, suffering from a mutation of a residue close to those affected in KID/HID syndrome, has no keratitis, only mild erythrokeratoderma, mild hypotrichosis, and brittle nails. Her phenotype is actually more reminiscent of the Clouston syndrome. Explaining the obvious differences between the phenotypes is in our view difficult if we do not accept that at least some residues of *GJB2* may have very specific roles in protein function that may not necessarily be related to gap junction assembly and function. Our results also point to an interesting difference between *GJB2* and *GJB6* with regard to their role in hearing loss. Although it was suggested that Cx26 and Cx30 form heteromeric connexons within the inner ear (Marziano *et al*, 2003), the Clouston syndrome is not associated with deafness. But a T5M mutation in *GJB6* does cause dominant non-syndromic hearing loss (Grifa *et al*, 1999). The *GJB2* mutations that are associated with skin symptoms then again all cause deafness. We have no explanation for this difference but as in the skin, transdominant effects on the expression of other connexins may be responsible for the hearing loss. *GJB2* may have a more important role in gap junction assembly in the inner ear. Alternatively, *GJB2* may have interactions with other proteins that are important for hair cell function that *GJB6* lacks. Recent reports of an interaction with the transcription factor YAF-2 support this notion (Rodina *et al*, personal communication, 2003). Specifically, YAF2 protein could be co-immunoprecipitated with aa161-226 C-terminal fragment of Cx26 *in vitro*. Confocal microscopy revealed co-localization of YAF2 and Cx26 on the plasma membrane of HeLa cells stably co-transfected with those genes. This finding suggests protein–protein interaction between YAF2 and Cx26 *in vivo*.

Further studies, for example *in vitro* experiments using expression vectors bearing mutated connexin genes, are urgently needed to understand the underlying mechanism of the presently bewildering variety of connexin26-associated disorders.

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